



Research article

Retention modes of manure-fecal coliforms in soil under saturated hydraulic condition

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ABSTRACT

Manures are important soil nutrient conditioners and source of several pathogenic bacteria that potentially contribute to groundwater and surface water pollution. The best management practices need a solid understanding of manure sources, concentrations, and strategies to limit the number of bacteria in natural soil environment. In this study, a series of soil column experiments were conducted to investigate how bacteria mobility can potentially be influenced by retention mechanisms while moving through undisturbed saturated soil. This was assessed by bacteria retention profiles and mobility indices including the maximum transported (C_{max-T}) and retained (C_{max-R}) concentrations, filtration coefficient (λ_f) and the maximum depth of bacteria transport (Z_{max}). Three different soil samples (sandy, loamy and silty clay loam) were enriched with three types of manures (cow, sheep, and poultry), placed on top of three soil columns (16 cm diameter, 30 cm height) with an equivalent of 10 Mg ha^{-1} (dry basis) summing up to a total of 36 columns. Leaching was performed under saturated steady-state conditions (i.e., 1.62 cm min^{-1}) for a duration of 4 pore volumes. After percolation, soil columns were sliced into six 5-cm interval layers and slices were characterized for retained bacteria. Results showed irregular exponential or quasi-exponential bacteria retention profiles for cow and sheep manures, whereas uniform-shaped profiles occurred for poultry manure. The latter variant also switched to exponential shape for the sandy soil at the highest and lowest C_{max-T} and C_{max-R} values due to higher pore water velocity. The λ_f and the Z_{max} values were also found to be affected by soil texture and manure origin. The λ_f was higher for poultry manure due to higher free-cell transport of bacteria most probably induced by higher amount of soluble mobile components. However, the greater amounts of transported wooden materials released from cow and sheep manures acted as harbors for bacteria. Accordingly, the filtration rate decreased and tailing effects for bacteria transport increased. The results also suggest that the practices and strategies for using manures could be optimized according to the respective transport behavior to manage the bacteria retention with respect to soil and manure types to reduce soil and water pollution.

1. Introduction

Application of animal manures as a soil amendment is a common practice in agriculture for a cost-saving and efficient management way to recycle nutrients from animal wastes (Binh et al., 2008; Miller et al., 2015; USDA, 2009, 2014). Globally, $\sim 10^{11}$ tons of animal manures are routinely applied to the agricultural soil each year as a crop fertilizer (Yao et al., 2015). Land application is the preferred disposal method for most types of manures (Bicudo and Goyal, 2003). However, spread of manures leads to distribution of large numbers and species of

microorganisms into the soil (Guber et al., 2007). The amounts of manure bacteria are commonly high and this has limited the manure utilizations to agricultural land (Goss and Richards, 2007). Generally, the most common bacteria presented in the manures belong to faecal coliform so that they had been proved as an indicator for microbial water quality (Bradford et al., 2013; Weidhaas et al., 2014).

One key problem of field manure application is bacteria mobility according to the specific retention and transport mechanisms in soils (Bradford et al., 2013; Jamieson et al., 2002). The resident bacterial cells in the soils after manure application can increase the pollution

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risks of soil, water and agricultural productions, because bacteria generally can survive for long periods within the water-filled pore voids where bacteria colonies face optimum conditions of reproduction (Unc and Goss, 2004; Or et al., 2007). Therefore, the retention of bacteria in soil profiles can potentially be used a key parameter to monitor soil contaminations in the manure field management programs.

Systematic clean-bed studies (*i.e.* sand media) demonstrated that the shape of bacteria retention through soil profile depends beside other factors on the favorable and unfavorable attachment conditions between bacteria cells and porous media (Bradford et al., 2013). Previous studies have reported that the bacteria can be deposited in different shapes of hyper-exponential, exponential and uniform cell concentration profiles with soil depth (Bradford et al., 2002, 2003; 2013; Kim et al., 2009; Torkzaban et al., 2008). The hyper-exponential and exponential shapes indicate that most of the bacteria are retained close to soil surface where the bacteria are usually entering the soil after manure application. In this case, surface water like streams or lakes are more vulnerable to be directly contaminated by bacteria through sediment transport facilitated by surface run-off. The uniform depth profile of retained bacteria, however, illustrate an equal or higher risk of subsurface groundwater contamination compared to stream and lake waters (Bradford et al., 2013). Considering the field manure application practices, it should be taken into account that microorganisms are released together with the manure particulates which may support the coupled transport of microorganisms and manure components (*i.e.*, organic and inorganic colloids) and which makes prediction of their transport behavior even more complex (Blaustein et al., 2016; Guber et al., 2005, 2007; Shelton et al., 2003). This may increase or mitigate bacteria transport and retention risks through soil affected by other environmental factors (Guber et al., 2006; Martinez et al., 2014; Stocker et al., 2015). Up to now, however, a mechanistic understanding of the effects of manure types on microorganism retention and mobility in agricultural soils is still rather limited (Blaustein et al., 2016; Guber et al., 2005, 2007; Stocker et al., 2015; Weaver et al., 2016).

The maximum amounts of transported and retained bacteria are important to assess filter characteristics of the respective porous media (Guber et al., 2006, 2013; Martinez et al., 2014; Porter et al., 2016). The penetrating depth of bacteria cells in soil is also important for many processes, *i.e.* in the context of soil erosion, *i.e.* problematic situations may occur when the major cell mass remained close to the soil surface (Blaustein et al., 2016; Stocker et al., 2015; Weaver et al., 2016). It is also an indicator for attachment or transport of bacterial cells in the soil for bioaugmentation purposes (Dong et al., 2002) and may be rated as important for affecting bacteria growth conditions (Stocker et al., 2015; Unc and Goss, 2004; Weaver et al., 2016).

We hypothesize that the transport and retention of bacteria through soil strongly depends on the physical behavior of the applied manure in terms of solid and soluble material contents in conjunction with environmental conditions. In this study, retention mechanisms of fecal coliforms was investigated for three textures including sandy, loam, and silty clay loam soil columns under saturated condition. The undisturbed soil columns were treated with poultry, cow and sheep manures in order to measure following parameters: (i) the retained viable bacteria in the soil profiles, (ii) the transport parameters indicating maximum transported and retained concentrations (C_{max-T} and C_{max-R}) and the filtration coefficient (λ_f) and the maximum depth of transport (Z_{max}). Based on this evaluation we discuss risk potential of these wide-spread manure sources.

2. Material and methods

2.1. Soil columns and manure sample preparation

Thirty-six undisturbed soil cores with sand (Typic Xeropsamment), loam (Typic Haploxerept) and silty clay loam (Typic Haploxerept) textures were sampled using soil columns with inner diameter of 16 cm

and 35 cm length) from an agricultural field site in Marvdasht, 45 km north of Fars province in Iran (29°52'27"N 52°48'09"E). The soil columns were filled with 30 cm of soil, leaving a headspace with 5 cm height to serve as water reservoir during leaching experiments (Mosaddeghi et al., 2009; Sepehrnia et al., 2014, 2017). Data on soil textures are presented in Sepehrnia et al. (2017) in greater details.

The sources of fecal coliforms were fresh cow (*Bos taurus*), sheep (*Ovis aries*) and chicken (*Gallus gallus domesticus*) manures. The manures were taken immediately after deposition (< 5 min) and then air dried in shade for 72 h and passed through a 2-mm sieve to obtain similar maximum particle size before using it for leaching experiments (Weaver et al., 2016; Sepehrnia et al., 2017). The particle size distributions (PSDs) (less than 2 mm) were determined by shaking 40 g dry manures for 5 min through a sieve-series using a sample divider machine (PT 100, Retsch, Germany) (Hafez et al., 1974). Further sieving was also performed using a sieve to separate manure particles larger than 150 μm under wet condition (Kemper and Rosenau, 1986). Geometric mean diameter (GMD) of manures was calculated using (Mazurak, 1950):

$$GMD = \exp \left[\frac{\sum_i^n w_i \log(\bar{x}_i)}{\sum_i^n w_i} \right] \quad (1)$$

where \bar{x}_i is the mean diameter of each size fraction, w_i is the proportion of the total sample weight occurring in the size fraction i .

Water repellency of manures was measured using water droplet penetration time test (WDPT), so that six drops of distilled water ($58 \pm 5 \mu\text{L}$ in volume) was placed on the manure surfaces either origin-or 2-mm bulks from a standard height of 1 cm above the surface, and the time needed for the drop to penetrate into the manures was recorded (Lichner et al., 2012). Further physical, chemical and biological properties including saturated hydraulic conductivity (K_s), saturated water content (θ_s), pore water velocity (q/θ_s) (*i.e.* q steady-state flux density), porosity, pH, electrical conductivity (EC), cation exchange capacity (CEC), organic carbon (OC), influent (C_D) and, effluent (C) fecal coliform concentrations of the studied soils and manures are given in Sepehrnia et al. (2017).

2.2. Leaching experiment

The leaching experiments were conducted under saturated conditions where the soil columns were saturated by submerging the columns into tap water for 24 h (sand) and for 48 h (loam and silty clay loam). Thereafter, a steady water flux equal to the highest saturated hydraulic conductivity (Klute and Dirksen, 1986) reported by Sepehrnia et al. (2017) (*i.e.*, 1.6 cm min^{-1}) was conducted on all soil columns by a constant water head as upper boundary condition. This kept all soil types saturated (Sepehrnia et al., 2014, 2017). A negative pressure (*i.e.* seepage face; hSeep, Šimůnek and van Genuchten, 2008) was applied at the bottom of loam and silty clay loam columns (*i.e.* 1–2, and 2–4 kPa, respectively, Sepehrnia et al., 2017) as lower boundary condition using a vacuum pump. For sandy soil, however, the steady-state flow of lower boundary condition was established without using the vacuum pump due to natural features of its coarser textures and gravity drainage.

Once steady-state flow was established, the air-dried sieved (2-mm) manures were separately applied on the top of the soil surfaces in the columns with an equivalent concentration of 10 Mg ha^{-1} (dry basis). The leaching of the soil amended-manures were continued for 4 pore volumes. Sixteen effluent samples were collected from each column during the leaching using sterile syringes at 0.25 pore volume intervals to evaluate the concentration of effluent fecal coliforms (Sepehrnia et al., 2014, 2017). The leaching experiments were performed separately for the control columns without manure and the manure-treated columns, all in triplicate. All leaching experiments were conducted at room temperature ($25 \pm 2^\circ\text{C}$).

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